LARVAL TREMATODES FROM AUSTRALIAN FRESH-WATER MOLLUSCS

Part XV

Cercaria velesunionis n. sp.

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SUMMARY

Cercaria velesunionis, a gasterostome, is described from the fresh-water mussel, *Velesunio ambiguus*. (It is possible, but is not considered likely, that *Alathyria jacksoni* can also serve as host.) It is not a common parasite, having been found in only 16 of 1818 mussels examined.

It has a different excretory formula, $2\{(2+2)+(2+2)\}$, from gasterostome cercariae for which the excretory formula has been described. Since it cannot be compared fully with cercariae for which the excretory formula is not known, it is assigned to a new species.

The cercaria has been found, experimentally, to encyst in the fish *Gambusia affinis*, *Carassius auratus*, *Oryzias latipes* and *Galaxias* sp.

Adult gasterostomes have never been identified from fresh-water fish examined in this department. These are *Maccullochella macquariensis*, *Plectroplites ambiguus*, *Pseudaphritis urvillii*, *Tandanus tandanus*, *Therapon bidyana*, *Fluvialosa richardsoni* and *Macquaria australasica*.

Immature gasterostomes, probably of the same species as *Cercaria velesunionis*, were found in four *Percalates colonorum*.

Until the adult trematode is found, *Cercaria velesunionis* cannot be assigned to a genus.

Type material has been deposited in the South Australian Museum.

Fielder, in a paper read before the Field Naturalists' Club of Victoria on May 10th, 1896, recorded as "the most interesting find of the month", "curiously modified fluke embryos in the fresh-water mussel (*Unio australis*)". From Fielder's short description, without figures, these were obviously gasterostome cercariae, and it would seem likely that it is the first record of *Cercaria velesunionis*, which is described in this paper. I know of no other records of cercariae from fresh-water mussels in Australia. McMichael and Hiscock (1958) list *Unio australis* Lam., of Smith, 1881, as a synonym of *Velesunio ambiguus*; *Unio australis* Lamarck, 1819, they assign to the genus *Hyridella*, subgenus *Hyridella*.

In this paper is given a description of the sporocyst, cercaria, cyst and metacercaria of *C. velesunionis*. Only immature adults have been obtained, however. Mature adults could not be obtained with attempted infections of three *Carassius auratus*.

Since June, 1937, gasterostome cercariae have been identified in 16 of 1818 fresh-water mussels collected in the River Murray between Tailem Bend and Morgan. They have been found on only seven occasions, in 1 of 3, 2 of 6, 2 of 16, 5 of 31 and 2 of 106 mussels from Tailem Bend, in 3 of 25 from Morgan, and in 1 of 70 from Teal Flat.

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Mussels from the River Murray were always identified as *Hyridella australis* (Lamarck). However, in 1958, McMichael and Hiscock published a "Monograph of the fresh-water mussels (Mollusca : Pelecypoda) of the Australian region". According to this, there are two species of mussel in the Tailem Bend-Morgan stretch of the Murray, *Velesunio ambiguus* (Philippi) and *Alathyria jacksoni* Iredale. Of the sixteen infected mussels collected, ten are still available and are now identified as *Velesunio ambiguus*. My impression is that probably no *Alathyria jacksoni* (fully grown shells of which are bigger and heavier than those of *Velesunio ambiguus*) have been found infected. The matter, however, must remain in some doubt. In the most recent collection, of 70 mussels from Teal Flat, only about 15 were *V. ambiguus*, but it was from these that the single infection was found.

The cercariae tend to lie at the bottom of the dish in which the mussel is isolated. Here, as observed with a dissecting microscope, they contract and expand the body, and may extend the furcae to great lengths and then contract them to become quite short. (These movements are not necessarily synchronised.) If the water in a beaker containing the cercariae is disturbed slightly, they can just be seen hanging suspended in the water by the two outstretched furcae. The body occasionally contracts, and, not always at the same time, one or both furcae contract also, but the cercariae do not change their position in the water by this means, and have never been seen to swim.

The following measurements were made on 20 cercariae which had been fixed by adding an equal volume of boiling 10 p.c. formalin to the water containing them. Body length 142-222 μ (average 175 μ); greatest width of body 55-97 μ (average 70 μ); length of anterior organ 37-71 μ (average 50 μ); breadth of anterior organ 25-43 μ (average 31 μ); width of tail stem (i.e. transverse diameter) 84-116 μ (average 100 μ); depth of tail stem (vertical measurement) 41-70 μ (average 45 μ). The furcae are too coiled in formalinised specimens to permit of measurement. In one living specimen the furcae were drawn out in a straight line by a current of water, and at their greatest length measured 2·4 mm.

The preceding measurements were taken from cercariae collected in late 1959 and early 1960. A collection made at Morgan in February 1956 from three mussels comprises cercariae noticeably larger. Measurements of 20 of these fixed in the same way as the later infections are: body length 175-350 μ (average 300 μ); greatest width of body 72-115 μ (average 91 μ); width of tail stem 58-130 μ (average 84 μ); depth of tail stem 44-65 μ (average 51 μ). Although the difference in size probably has no real significance, the description is based on cercariae from the 1959-1960 collections; in a few instances, information about the 1956 material has been included, but where this is so the date is stated.

The body of the cercaria is set with rows of fine spines which are quite prominent anteriorly but are very inconspicuous towards the posterior end of the animal. The anterior organ is quite well-developed; its cavity is comparatively small, elongated in the antero-posterior axis, and is lined with closely-set spines, which, though small, are much longer than those on the surface of the body. This region is more or less eversible, and when everted gives the appearance of a small spine-covered snout protruding anteriorly. In one favourable specimen five pairs of gland cells were seen in the anterior organ (Fig. 1). In arrangement these resembled those figured for *Cercaria scioti* by Woodhead (1936, plate LIX, fig. 1), with the exception that they did not rest on the basement membrane of the organ, but were situated more anteriorly. The ducts

opened into the inverted part of the organ, which in this region appeared to be divided in two as in Woodhead's figure.

The mouth opens behind the middle of the body, nearly as far back as the level of the posterior third. There is a large muscular pharynx, an oesophagus, and a gut, which varies in shape from spherical to oval.

Gland cells scattered throughout the body show up after staining with neutral red. They do not appear to have any recognisable arrangement, but the ducts pass forward anteriorly.

The flame cell formula is $2\{(2+2)+(2+2)\}$. The most posterior pair of flame cells lies near the hind end of the body, and in much flattened specimens the last flame cell may appear to lie in the tail stem itself. The bladder is I-shaped; the anterior and posterior collecting tubes join the main excretory tube at the level of the mouth, and at the point of their union is a distinct dilatation of the tube (Fig. 1). The positions of the flame cells vary slightly in the cercariae examined, probably due to the relative compression of the various parts. The bladder opens at the posterior end of the body.

The base of the tail has the appearance of a cushion which consists of two regions. The upper segment is relatively clear and contains an extension of the bladder, which contracts at times so that the cavity disappears completely. The cytoplasm of the lower segment is filled with fatty globules. Some part of the base of the tail has a sticky secretion, for the animal can attach itself to a glass surface by this region. In one specimen, in which the body was quite free, the animal was attached by the base of the tail; it was impossible to dislodge it with currents of water directed from a fine pipette, and needles were required to free it. Dawes (1946, p. 456) stated that, according to Wunder (1924), the "rudimentary tail" of *Bucephalus polymorphus* secreted a viscid material which served to fasten the cercaria to the body of the fish intermediate host.

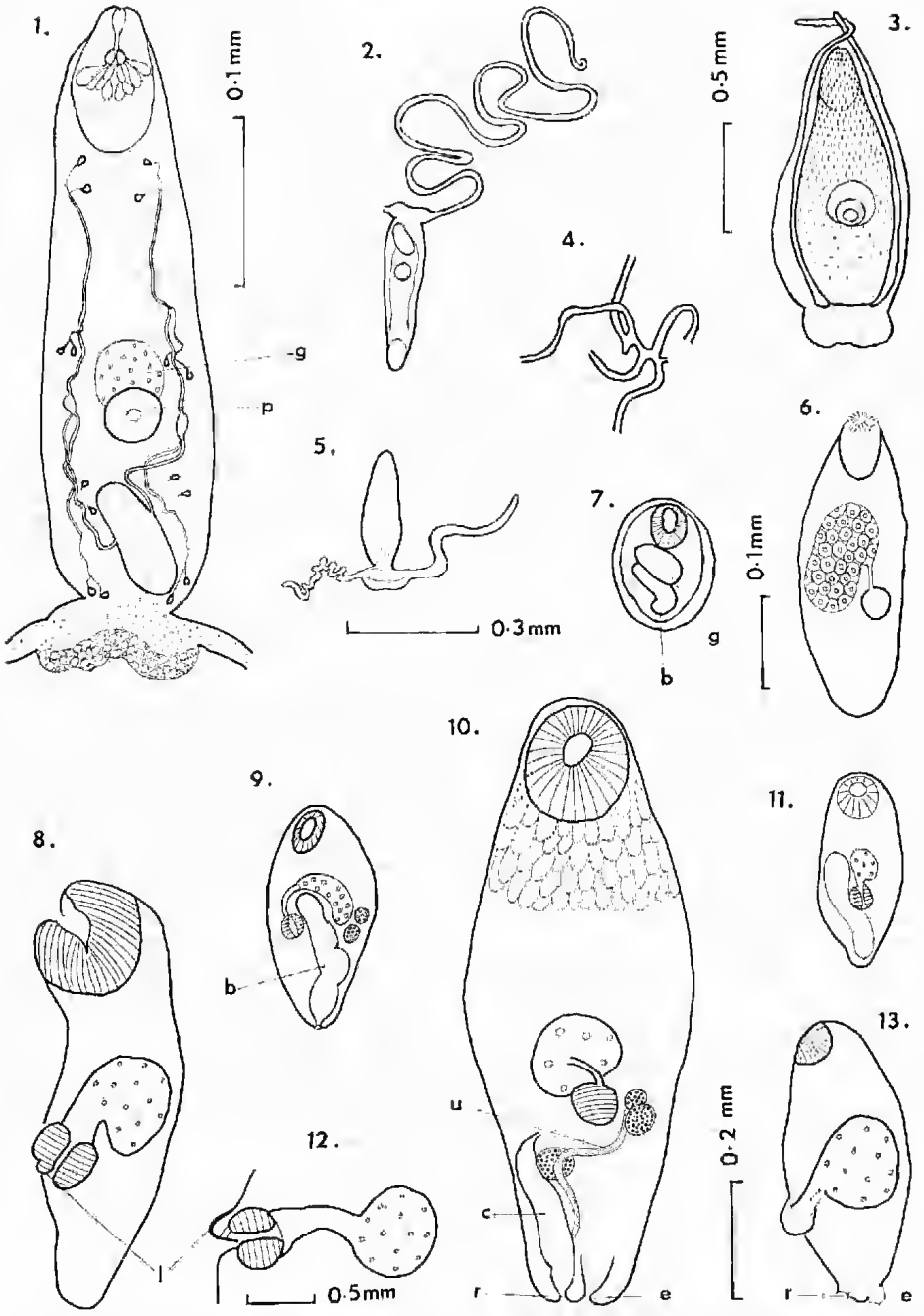
The furcae leave the outer margins of the upper segment of the base of the tail. They, too, are sticky; they become attached to fine needles used in handling the cercaria, and tend to break if pulled, rather than become detached. Even in formalinised cercariae, the furcae are sticky; it is difficult to clear them of any débris adhering and at the same time prevent them from breaking with any traction.

Woodhead (1936) made tests with live fish and stated that, with *Cercaria scioti*, *C. basi* and *C. argi*, the long furcae became entangled on the edge of a fin, and the tail stem functioned as a holding organ by becoming firmly attached, with the posterior portion in contact with the scales of the fish.

The furcae have extraordinary powers of extension. When contracted, they may be little more than the length of the body (Fig. 3), but when extended may be ten times as long. The contracted condition is not, apparently, the normal one; when the animal is suspended in water the furcae are elongated most of the time, and in formalinised material contracted furcae are not seen.

The Sporocyst

The sporocysts form a thick mass in the digestive gland, and are scattered through the region of the gonad and heart. They branch, apparently very freely, but it is difficult to obtain a sporocyst that one is sure is complete. Formalinised material is brittle and breaks easily, and it is hard to dissect out living sporocysts intact. Living sporocysts contain many cercariae, the furcae of which may protrude from the broken ends of the sporocyst to a great distance. No flame cells were seen in the sporocyst walls.



Figs. 1-3, 5, 6. *Cercaria velesunionis;* 1, compressed; 2, 3, 5, in different attitudes; 6, showing gut. Fig. 4. Sporocyst. Fig. 7. Cyst. Fig. 8. Young gasterostome from Murray Perch. Balsam mount. Figs. 9-13. Metacercaria of *C. velesunionis*; 9, 11, 13, different aspects; 10, compressed; 12, alimentary canal from lateral view, showing lip. Fig. 8 is to the same scale as Fig. 12. Figs. 2, 7, 11 are to the same scale as Fig. 5. Figs. 4, 9, 10 are sketches.

b, extretory bladder; c, cirrus sac; e, excretory pore; g, gut; l, lip; p, pharynx; u, uterus; r, reproductive aperture.

The Cyst

The cercariae have been found, experimentally, to encyst in the aquarium fish *Gambusia affinis*, *Carassius auratus*, *Oryzias latipes* and the native fish *Galaxias* sp. Gasterostome cysts were found as a natural infection in three *Carassius auratus* from Tailem Bend in 1937; these appear to be the same as the cysts obtained experimentally.

The cysts may be found in great abundance in the infected fish. In 1956, when detailed dissections were made, they were more numerous in the head region than elsewhere in the body. In one fish 103 cysts were recovered from the head region and only four in the rest of the body. In another there were 25 in the head region; 9 in the tail and 20 in the tissue between the fin rays of other fins. However, in heavily infected fish (1959-1960) there were literally hundreds of cysts in the tail region. (The rest of the body was not dissected, but was used in feeding experiments to try to find the adult stage.)

The cyst walls are thin and the metacercariae so active that the cysts continually change shape, from oval to circular, to pear-shaped, etc. The cyst wall breaks extremely readily, and excystment takes place spontaneously shortly after the tissues of the host are dissected apart. A few hours afterwards it is rare to find even one intact cyst. In order to collect sufficient cysts for measuring, it was found necessary to transfer them into formalin as soon as they were dissected out. (Table 1.)

The Metacercaria

Metacercariae dissected into 0·65 p.c. saline live for several days at 4° C., and up to twenty-four hours at room temperature.

Two fish, *Gambusia affinis*, were infected four and eight weeks respectively before they were killed; the metacercariae from these were accidentally mixed, but could be separated into two distinct ranges of size. The smaller size is presumed to comprise the younger metacercariae (see Table 1 for measurements of the two groups).

The body is spined all over; the spines are relatively large and obvious in the anterior half of the body, but become smaller and are very inconspicuous posteriorly. After staining with methylene blue, granular subcuticular cells of irregular shape can be seen (though they do not take up the stain) scattered throughout the body; no nuclei are visible. After neutral red a mass of gland cells shows up behind the anterior sucker. Individually, these cells are not very distinctly defined, but the area as a whole is very definite (Fig. 10). It extends behind the anterior sucker to a distance roughly equal to the length of the sucker.

The anterior organ has increased greatly in size and has much more the appearance of a normal sucker than was the case in the cercaria. It has no lobes or appendages.

The mouth opens into a muscular pharynx which is followed by a distinct oesophagus; this is quite contractile, and opens into the sac-like gut. From the lateral view, a definite lip on the anterior border of the mouth is seen (Fig. 12). The gut, which occupies, roughly, the middle of the body, is generally rounded, but sometimes elongated. It often shows as a conspicuous yellow mass because of its bright yellow contents, which consist of a somewhat viscous liquid in which are refractile globules of various sizes. The mass of the gut contents stains deeply with neutral red.

The flame cell formula is the same as in the cercaria. The excretory bladder is elongated; it extends anteriorly beyond the mouth, and posteriorly opens on the end of the body close to the excretory pore. Very often it lies diagonally

TABLE 1.

Metacercariae and young adults of *Cercaria velesuntonis*.

N.B. Numbers given in brackets below the measurements indicate the number of organs measured.

				Length		Breadth		Anterior organ				Pharynx			
								Length		Breadth		Length		Breadth	
		Age weeks	Number measured	Range mm.	Average mm.	Range mm.	Average mm.	Range mm.	Average mm.	Range mm.	Average mm.	Range mm.	Average mm.	Range mm.	Average mm.
Cyst (1959-1960)	In formalin	8	20	·217—·334	·280	·145—·232	·180								
Cyst (1956)	In formalin	up to 16	10	·235—·341	·313	·200—·247	·226								
Metacercaria	In formalin	4	20	·232—·319	·255	·131—·174	·152								
Metacercaria	In formalin	8	20	·334—·464	·390	·116—·203	·157								
Metacercaria	Living	?	10	·309—·463	·382	·130—·247	·189	·080—·114	·098	·074—·108	·090	·040—·059	·053	·037—·062	·049
Metacercaria	Living	6—7	10					·074—·108	·090	·080—·120	·098	·037—·062	·049	·040—·059	·052
Metacercaria (1956)	Lactophenol	?						·059—·093	·077 (10)	·049—·071	·062 (10)	·034—·037	·036 (5)	·028—·037	·033 (5)
Young adults from carp	Flattened: in cedar-wood oil		1		·263		·136		·068		·068		·034		·037
			1		·253		·127		·068		·068		·037		·034
Young gasterostomes from *Percalates colonorum*	In Canada balsam		17	·213—·367	·300	·083—·142	·101	·068—·099	·083 (8)	·068—·093	·083 (8)	·037—·046	·041 (4)		·046 (4)
	Flattened: in glycerine		1		·355		·142		·093		·093				
			1		·343		·142		·096		·083				

across the body (Fig. 11). In freshly dissected specimens it is filled with dark excretory granules, tiny to small in size, some of them compound. These are extruded readily through the excretory pore.

The anlagen of the reproductive organs are fairly well defined (Fig. 10). There are three small rounded masses of cells which are evidently ovary and testes. The ovary is smaller than the others, and lies slightly anterior to the anterior testis; from it a ribbon of cells which is presumably to become the uterus, winds backwards between the diagonally placed testes, and then runs alongside the cirrus sac, which is a large organ lying to the side of the bladder at the posterior end of the body. The genital pore opens at the posterior end of the body, close to the excretory pore. Vasa deferentia are not seen, nor is there any trace of vitellaria.

The Adult

A laboratory-raised carp, *Carassius auratus*, was fed over a period of five weeks with 15 *Gambusia affinis* and one small carp, all of which had been exposed to infection with *Cercaria velesunionis*. Some of these small fish were partly dissected before being fed to the carp, and it is estimated that it was probably given two or three thousand cysts. The carp was killed five days after the last small fish had been fed to it. Two young gasterostomes were recovered from its intestine. Although these had had at least five days (and could have had as much as five weeks) in the gut, they showed little difference from the metacercaria of *C. velesunionis*. (See Table 1 for measurements.) The guts of the two specimens measured ·068 x ·068 mm. and ·068 x ·056 mm. respectively.

Two more carp were fed with an unknown number of cysts; they were killed 18 and 27 days respectively after the latest feeding. Neither yielded gasterostomes on dissection. The failure to establish infections indicated that *Carassius auratus* is not a suitable host for the species.

Adult gasterostomes have not been found in any fresh-water fish examined in this department. These include 31 Murray cod (*Maccullochella macquariensis*), 96 callop (*Plectroplites ambiguus*), 33 congolli (*Pseudaphritis urvillii*), 52 catfish (*Tandanus tandanus*), 10 Murray bream (*Therapon bidyana*), 4 bony bream (*Fluvialosa richardsoni*) and 12 Macquarie perch (*Macquaria australasica*).

Immature gasterostomes, however, were found in four of seven Australian perch, *Percalates colonorum*, taken from the River Murray at Swan Reach in September 1937. (They have not been found in nine *P. colonorum* collected from the River Murray since that time.) In size and general appearance, and in the presence of a lip, these young flukes are very similar to the metacercariae of *Cercaria velesunionis*. They are regarded as belonging to this species.

DISCUSSION

Cercaria velesunionis is regarded as a new species. It cannot be identified with cercariae which have been described without details of the excretory system, and it differs from other gasterostome cercariae in which the excretory system has been described (*C. elegans* Woodhead, 1930; *C. papillosus* Woodhead, 1930; *C. scioti* Woodhead, 1936; *C. argi* Woodhead, 1936, and *C. losi* Woodhead, 1936) in having an excretory formula of $2\{(2+2)+(2+2)\}$. Hopkins (1956) pointed out that in the taxonomy of Bucephalidae, the structure of the excretory system had not been given phylogenetic significance. The excretory formula, $2\{(2+2)+(2+2)\}$, was found in species of three different genera, while four different formulae were found in one genus (*Rhipidocotyle*).

Hopkins was of the opinion (and many other workers must agree with him) that no natural classification can be made without taking the excretory system into consideration. The nature of the anterior attachment organ, however, has been regarded as of great taxonomic importance in the Bucephalidae. In 1954, Hopkins (p. 355) pointed out that the cercariae of *Bucephalus elegans* Woodhead, 1930, *Rhipidocotyle papillosum* Woodhead, 1929, and *R. septpapillata* Krull, 1934, the only bucephalids whose life cycles had been established by experiment, did not show any signs of the papillae or hoods which distinguished the adults from the forms which Nicoll (1914) assigned to *Bucephalopsis*. He stated that, so far, there was no way of telling which genus of the Bucephalidae a given cercaria belonged to, until the life cycle had been worked out by experimental infection. To my knowledge, no life histories have been described since Hopkins' statement.

Until the adult form is found, it is not possible to assign *Cercaria velesunionis* to a genus.

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